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CERTIFICATE

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Title of the invention:	Agent for Inhibiting Membrane Virus Reproduction, Method for the Production thereof, Pharmaceutical Composition and Method for Inhibiting Viral Infections
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AGENT FOR INHIBITING MEMBRANE VIRUS REPRODUCTION, METHOD FOR THE PRODUCTION THEREOF, PHARMACEUTICAL COMPOSITION AND METHOD FOR INHIBITING VIRAL INFECTIONS

The present invention relates to the pharmaceutical industry and more particularly to the provision of an agent for inhibiting membrane virus reproduction.

The present invention relates to compounds and pharmaceutically acceptable salts thereof which inhibit the reproduction of membrane viruses, such as human immunodeficiency virus (HIV), type 1, herpes simplex virus (HSV), type 1, hepatitis C virus (HCV), genotype 1c.

At present investigations are under way for developing the therapy and methods of treating viral infections, especially those provoked by HSV, HCV and HIV/AIDS, and the AIDS-associated complex. AIDS patients whose immune system is disturbed suffer from numerous opportunistic infections caused by such pathogens as Pneumocystis carinii and Candida albicans, HSV, HCV, cytomegalovirus (CMV), or from some kinds of tumors (Kaposi's sarcoma), which become the direct cause of death. A method for treating AIDS is not known, and the current therapy in most cases is employed without sufficient proofs of its efficiency and has unfavorable side effects.

HIV is characterized by a high genetic and, consequently, antigenic variability. HIV strains obtained even from one and the same patient but at different stages of the patient's disease may differ in the antigenic properties and nucleotide sequences. A difference of strains in different climatogeographic zones. This complicates the chemotherapy, immunotherapy and vaccinal prevention of AIDS.

Problems in treating HSV infections arise because of the ability of these viruses to persist in a latent or dormant form. When the primary infection abates or retreats, the virus, in the main, remains in the latent form in sensitive nerve ganglia which innervate the site of the primary infection. The decisive period of latency is unknown; besides, this period can be disturbed by overheating, cooling, solar irradiation, hormonal and emotional deviations or by immunosuppressive means, leading, in the main, to reinfection.

Most of the antiviral means employed so far for the treatment of HSV infections have been substances which interfere in the viral DNA synthesis. These substances, include iodohydroxyuridine, cytosine, arabinose, adenine arabinoside and trifluoro-

thymidine. Such substances interfere also in similar host cell functions, and this involves cell toxicity problems and, as a consequence, makes their systematic use in humans impossible. At present the main drug for treating infections caused by HSV is acyclovir which has strong antiviral activity and low toxicity. However, its weak solubility and the appearance of drug-resistant viruses restrict the application of this drug.

The chemotherapy of AIDS is associated at present with the creation and use of reverse transcriptase inhibitors and also of HIV protease inhibitors.

HIV reverse transcriptase (revertase) inhibitors are either of nucleoside nature: Zidovudine (AZT, Retrovir), Epivir 3TC (Lamivudine), Videx (ddl, Didianosine), Hivid (ddc, Zalcitabin), Zerit (d4T, Stavudine), Abacavir (ABC, Ziagen), Combivir (Zidovudine + Epivir), Trizivir (Abacavir + Epivir + Zxidovudine) or of non-nucleoside nature: Delavirdine (rescriptor), Nevirapine (Viramune), Efavirenz (Sustiva), or of nucleotide nature: Tenofovir, Viread. Russian-made preparation Phosphazide was registered in Russia. These preparations are toxic for a macroorganism as well, because they interfere in genomic structures. Reverse transcriptase synthesizes viral DNA throughout the period of the disease, and therefore it is necessary to use HIV revertase inhibitors for life.

At the present time HIV protease inhibitors are represented by several preparations: Saquinavir (Invvirase), Indinavir (Crixivan), Ritonavir (Norvir), Nelfinavir (Viracept), Amprenavir (Agenerase), Kaletra (Lopinavir + Ritonavir), HIV protease is responsible for the ripening (processing) of viral proteins. Disturbance of the glycoprotein processing leads to the inability of HIV varions to attach to the CD4 cell.

At present there exists the term "third line therapy" for characterizing the treatment of HIV/AIDS patients in which the pathogen proved to be resistant to at least one drug from each class of the preparations, or in which the treatment with the use of two different schemes of therapy proved to be ineffective. Such highly active antiretroviral therapy (HAART) is also denoted by the terms "mega-HAART" or "giga-HAART". The third line therapy includes using simultaneously four antiretroviral preparations with different HIV reproduction blocking mechanisms: nucleoside and non-nucleoside reverse transcriptase inhibitors and protease inhibitors It is more complicated to prognosticate possible negative interactions of various drugs; as a result, the probability of the development of side reactions and complications sharply increases. In such cases constant monitoring of drug concentrations in blood serum is required, this being a costly procedure. The necessity of complementing the therapeutic course with preparations suppressing the activity of agents causing concomitant infections makes the process of treatment still more complicated.

The known medicinal preparations make it possible to control the course of the disease, but not to cure AIDS patients. The creation of drugs which can cure or at least provide better protection from the lethiferous virus is continued and is associated both with seeking new compounds capable of inhibiting the virus reproduction with the help of known mechanisms and with developing new approaches to solving the problem.

The range of preparations for treating HIV/AIDS patients is broadening. At present the following preparations are in the stage of clinical studies: Emtricitabine, DAPD (nucleoside analogs), Kapravirin, TMC-120 (non-nucleoside analogs). Attention is attracted also by such new protease inhibitors as Atazanavir (Erivada), Tipranavir, Mozenavir. Investigations are carried out with a view to creating preparations of absolutely new classes: interase inhibitors (S-1360), and also fusion inhibitors (Pentafuside (T-20 or Fuzeon)), T-1249, PRO-542, and chemokine receptor inhibitors (SCH-C and PRO-140). However, the results of tests are ambiguous. For instance, the SCH-C preparation caused an increase of the QT interval in the ECG of healthy individuals under test upon maximum dose administration, this being indicative of possible cardiological complications. Fusion inhibitors are polypeptides: T-20 consists of 36 natural amino acid fragments, T-1249 consists of 39 fragments. Use of these preparations is limited to intravenous administration; as a result of administering T-20, in some patients subcutaneous nodules were formed, occasionally subcutaneous infections and abscesses were noted.

The majority of the known most dangerous viruses: HIV. HSV, HCV, CMV, influenza virus are typical representatives of membrane viruses. Infection of a host cell with membrane viruses is initially based on the interaction of various receptors on the surface of the host cell with virus glycoproteins. Than the viral and cell membranes fuse together and the virion contents flow into the host cell cytoplasm. An interference in the formation of the virus membrane protein might preclude the initial interaction of the virus and the host cell and their subsequent fusion, and also inhibit the formation of varions. In RU 2196602 it is shown that under the effect of fulllereneaminocaproic and butyric acid salts there took place suppression of the replicative activity of CMV owing to the inhibition of the late gB structural protein.

High-polymer polyanionic natural compounds deserve special attention: peptidoglycans, dextrans, polysaccharides, etc. These compounds are low-toxic and capable ob adsorbing viral particles and serve as a xenobiotic "trap" of HIV varions. These substances inhibit the formation of syncytia, but direct effect of these drugs on the infectiousness of virus was not established (European Applications 04065512 and 0467185). It is suggested

that sulfonated polycarbamides (RU 2160746) with a molecular weight of from 2000 to 4000 suppress the HIV, HSV and HCV activity by the following mechanism: anionic groups of synthetic oligomers bind to the virus and/or cell membrane and thereby interrupt the virus ability to replication.

The known drugs, in the main, inhibit one of the specific functions of virus. In WO 95/199491995 the possibility was shown for the first time of acting with one substance on two targets: on protease and HIV reverse transcriptase. In RU 2196602 a method for the simultaneous inhibition of HIV infection and CMV infection was proposed for the first time. The inhibition was carried out by the mechanism of blocking the active site on the molecule of protease and reverse transcriptase and of the late gB CMV human structural protein. In both patents fullerene derivatives were used.

Recent attention has been given to the biological activity of fullerenes in connection with the possibility of using them for combating viruses. The main obstacle on the path to creating medicinal preparations is connected with the insolubility of fullerenes in water, which hampers their direct administration into human organism.

Methods of preparing water-soluble forms of fullerene through the formation of an adduct with polyvinyl pyrrolidone are known (Kiselev O.I. et al. // Mol. Materials, 1998, vol. 11, p. 121; Piotrovsky L.B. et al. // ibidem, 2000, vol. 13, p.41). Its effectiveness against influenza virus of type A and B is shown.

Also known is a method of preparing fullerenes, which comprises mixing fullerenes predissolved in an organic solvent with a polymeric matrix in chloroform, evaporating the mixture under vacuum until the solvents are completely removed, dissolving the resulting complex in a phosphate-salt buffer (pH 7.4—7.6), followed by ultrasonic treatment of the product (RU 2162819, 02.10.01). Membrane cephalins are used as the water-soluble matrix.

The products prepared as a result of such modifications are unstable compounds with limited shelf life.

A promising trend is the preparation of water-soluble of fullerene derivatives by chemical addition of various radicals thereto. The prior art most relevant to the present invention are compounds and methods of preparing thereof described in patents WO 95/19949 C07C 49/223, 1995, RU 2196602, RU 2124022.

Known in the art is a compound containing a water-soluble fullerene derivative of the general formula $C_{60}X$ = $HOC(O)(CH_2)_2C(O)NH(CH_2)_2$ (WO 95/19949, C07C 49/23, 1995). The substituents are any alkyl or aryl-alkyl substituents, in particular those which

are substituted with nitrogen or oxygen, having from 1 to 20 carbon atoms. However, this compound has low water-solubility, equal to 1 mg/ml, and the method of preparing it is complicated.

In RU 2196602 a method is proposed for inhibiting HIV reproduction and CMV infections with the help of compounds based on the amino acid and dipeptide derivatives of fullerene. Sodium salts of fullerene-monoamino-caproic acid and fullerene-monoamino-butyric acids are used as the fullerene amino acid derivative.

The compound closest in terms of the technical essence and the technical result is the compound N-(monohydro)-fullerene-amino-caproic acid $HC_{60}NH(CH_2)_5COOH$ (RU 2124022).

For preparing this compound, to a solution of fullerene in o-dichlorobenzene an aqueous solution of potassium salt of amino-caproic acid and 18-crown-6 are added. The reaction mass is stirred for 6—8 hours at 60°C. Then the solvents are distilled off, the residue is treated with a saturated solution of potassium chloride, and the residue of the fullerene derivative is washed with water. The yield of the target product is quantitative. The obtained N-(monohydro)-fullerene-amino-caproic acid is soluble in dimethyl-sulfoxide, dimethylformamide, and pyridine.

This synthesis is disadvantageous in that the conditions of the reaction of fullerene C_{60} and potassium salt of aminocaproic acid in a two-phase system lead to an increase of the process time; besides, 18-crown-6 used as a solubilizer is expensive.

The yield of the target product is small and does not exceed 5% of the weight of fullerene spent for the synthesis, and the properties of the products are non-reproducible.

Said process is disadvantageous in that part of the product is in the form of a water-insoluble compound.

In all the patents described earlier products of the monoaddition of amino acids and peptides to fullerene were obtained. However, fullerenes have a large number of equivalent reaction centers along double bonds, this providing an opportunity of polyaddetion products to be formed.

It is an object of the present invention to provide a product with a maximum possible number of substituted groups ensuring high solubility.

For solving the posed problem there is proposed a group of inventions united by a common inventive concept, an agent which is a compound comprising fullerene polycarboxylic anions, a method for the production thereof, a pharmaceutical composition comprising said agent, and a method for inhibiting the replication of membrane viruses.

As a result of the fullerene reaction with an amino acid salt in an organic solvent in the presence of a polyalkylene oxide, fullerene polycarboxylic anions of general formula I are obtained.

$C_{60}H_n[NH(CH_2)_mC(O)O^-]_n$,

where C_{60} is the fullerene core,

$NH(CH_2)_mC(O)O^-$ is the aminocarboxylic anion,

m is an integer from 1 to 5, preferably 3 and 5, most preferably 5,

n is an integer from 2 to 12, preferably from 4 to 6, most preferably 6.

The molecular weight is associated with the value m and n of the obtained compounds and is equal, respectively, to:

with n being equal to 6 and m being equal to 5, the molecular weight of the fullerene-hexaamino-caproic anion $C_{60}H_6[NH(CH_2)_5C(O)O^-]_6$ is 1500 g/mole.

For preparing the agent, into a solution of fullerene in o-dichlorobenzene (in toluene or in any other organic solvent) an amino acid is introduced in the form of a potassium or sodium salt, then a solubilizer is added. The order of introducing the amino acid and solubilizer into the reaction medium is of no importance, they can be introduced as a complex, having preliminarily intermixed them. As the solubilizer various polyalkylene oxides are used: polyalkylene glycols with a molecular weight of from 150 to 400 or higher (for instance, PEG-1500), and also polyethylene glycols having free end groups, as well as substituted ones (e.g., dimethyl ether of polyethylene glycol having the molecular weight 500).

For increasing the reaction rate, any strong reducing agent is added (alkali metals).

The fullerene/amino acid ratio is increased by more than 50 times. With the ratio smaller than 50, the obtained compounds have a lower solubility and higher toxicity.

The optimal synthesis temperature is +(60—80)°C.

Conversion to the desired pharmaceutically acceptable salt, especially to sodium or potassium salt, can be accomplished by treating the acid with a suitable base or by adding salt of a weak volatile acid. In particular, water-insoluble fullerene-polycarbloxylic acid is converted to more preferable pharmaceutically acceptable salts, such as sodium salt, which are soluble in water. Adding salt of a weak volatile acid is accomplished by treating solution with an alkali metal salt and a weak volatile acid. As the solution is concentrated by evaporation or lyophilization, the weak acid is removed and fullerene-polycarboxylic acids evolve in the form of their alkali metal salts. Sodium and potassium carbonate or bicar-

bonate are examples of salts of alkali metals and a weak volatile acid are sodium and potassium carbonate or bicarbonate.

The yield of the target product is more than 200% for the taken fullerene. The target product according to the present invention is characterized by constancy of the formulation, the content of the main substance in the target product is no less than 87.8%.

Compounds of formula (I) are dark-brown crystalline substances, unlimitedly soluble in water in the salt form and almost insoluble in water in the acid form. In the acid form these compounds are partially soluble in mixtures CH_3CN-H_2O 1:1 and i-PrOH-H_2O 1:1 and readily soluble in DMSO. When heated to 350°C, said compounds degrade without melting.

A particular feature of the structure of the obtained compounds is the presence of several carboxyl groups in the molecule, which, depending on the pH of the medium, are either in the salt form or in the acid form, displaying buffer properties. The pH of formation of complete salt is 7.9; the pH of formation of complete salt is 4.0, the acid, due to low solubility in water, forms micelles, a colloid, and precipitates.

The pH of transition of the claimed compounds to the dissolved state (formation of true solution) is 5.0—6.0. Mono- and disubstituted salts are formed.

At pH 5.0—7.9, due to hydrolysis, salts with different extent of substitution are in dynamic equilibrium.

The TLC was carried out on Merck $60F_{254}$ silica gel. The best results for the separation of the components were obtained with the system of eluents: EtOH-benzene-H_2O 4:1:1 (I) and 4:1:1.5 (II). In system (I) there were found 3 spots with R_f equal to 0.68, 0.37 and at the start; in system (II) 3 spots were found with R_f 0.82, 0.71 and 0.47, the latter belonging to the polar component. A sample with ninhydrin has shown the absence of compounds with a primary amino group in the product.

The obtained data indicate that the TLC-separated substances belong to different forms found in dynamic equilibrium, whose ratio depends on the pH and polarity of the solvent. This can occur in hydrolysis of incomplete salts of fullerene-polycarboxylic acids. The width of the chromatographic spot (area) of each component is indicative of the chemical (structural) homogeneity of the substance.

^1H and ^{13}C-NMR spectra of the solutions of compounds of formula (I) in deuterated solvents with different solvation capacities are recorded at 20°C on a WM-200 instrument with the working frequency 200.13 MHz for ^1H and 50.32 MHz for ^{13}C.

^{13}C-NMR (δ, D_2O): 25.2 ($\underline{C}H_2CH_2C(O)O$-), 25.4 ($\underline{C}H_2(CH_2)_2C(O)O$-), 26.8 ($\underline{C}H_2C(O)O$-), 69.5 ($\underline{C}H_2NH$), 130—160 ($\underline{C}_{60}$), 183.7 ($\underline{C}(O)O$-).

^{1}H-NMR (δ, CD_3OD): 1.16 d (1H, J = 6.0 Hz, -NH-), 1.26; 1.45 and 1.65 (3 m, 1H, 3H respectively, -$(CH_2)_3$-), 2.18; 2.23; (2 s, 0.2H, -NH...), 2.34 t (2H, J = 7.2 Hz, -$CH_2C(O)O$-), 2.94 br t (0.4 H, J = 6.0 Hz, J = 1.8 Hz, -NCH_2-), 3.6 m (1H, J = 1.8 Hz, J = 6.0 Hz, $C_{60}H$).

^{1}H-NMR (δ, DMSO-d6): 1.02 d (0.4H, J = 6.0 Hz, -NH-), 1.22, 1.32 and 1.52 (3 m, 6H., -$(CH_2)_3$, 1.90; 2.08 (2 s, 0.3H each, -NH...), 2.20 t (2H, J = 7.2 Hz, -$CH_2C(O)O$-), 2.68 q (2H, -NCH_2-), 7.46 m; 8.17 s (0.5H each, $C_{60}H$), 12.08 br s (1H, -C(O)OH)).

^{1}H-NMR (δ, D_2O): 1.25 m, 1.49 m (2H, 4H respectively, J = 7.8 Hz, J = 6.9 Hz, -$(CH_2)_3$-), 1.88 d, (0.1H, J = 1.6 Hz, -NH...), 1.89 d (0.1H, J = 5.5 Hz, -NH...), 2.05 d (0.1H,, J = 1.6 Hz, -NH ...), 2.24 t (2H, J = 7.3 Hz, -$CH_2C(O)O$-),2.82 br t (1.5H, J = 1.6 Hz, J = 7.5 Hz, -NCH_2-), 3.49 m; (1H, J=5.5 Hz, $C_{60}H$).

The following properties of the proposed compounds are conditioned by the presence of the fullerene core in the molecule. The multitude of isolated multiple bonds allows one to regard fullerene as a polyolefin system. Addition via multiple bonds is most typical of fullerene. It easily adds nucleophiles and free radicals, which makes it possible to use such substances as antioxidants.

The technical result of the invention consists in the provision of a novel class of compounds: fullerene-carboxylic anions of general formula (I) by the nucleophilic addition of two and more amino acids to fullerene via several double bonds.

These compounds have new properties:

- unlimited solubility in water,
- high bioavailability,
- high efficiency of action on infected cells,
- low toxicity.

It is shown that the antioxidant activity of the claimed compounds depend only little on their concentration. Upon 40-fold increase of the concentration — from 5 to 200 μg/ml — the antioxidant activity increased from 22.78 to 32.21 percent. Hence, the compounds even in low concentrations operate with maximum possible efficiency.

The antiviral activity of the agent was studied with regard to HIV, HSV, hepatitis C.

The compounds of formula (I) in the concentration of 1 μg/ml provided full protection of reinoculated lymphoblastoid human cells MT-4 against viral cytopathic action (VCA) of HIV-1, taken in a dose of 100 TCD_{50} to 7 of follow-up after the infection of cell cultures. At the concentration of the preparation of 10 μg/ml, disappearance of the virus (antigen p24) takes place in the culture medium. In these concentrations no cytotoxic action of the preparation on cells was revealed

The compounds of formula (I) in the concentration of 10 μg/ml provided full protection of the cells of the reinoculated culture of African green monkey kidney cells (VERO) and of human embryo fibroblasts (M-21) against the cytodestructive action of herpes simplex virus (HSV-1), taken in the dose of 100 TCD_{50} 48 hours after the infection of the cell cultures. During the same period of time in the control infected cell cultures which had not undergone the action of the claimed compounds 100% death of the cells took place.

The compounds of formula (I) in the concentration of 10 μg/ml introduced at the moment of infection provided full protection of the reinoculated cell culture of human adrenocarcinoma SW-13 against the cytodestructive action of hepatitis C virus (genotype 1b) taken in a dose of 100 TCD_{50}. The viability of infected cells remained on the level of non-infected control. There was established complete absence of residual infectiousness of the virus on the 3rd day after the infection of cells with the use of the proposed compounds in the concentration of 100 μg/ml; in the concentration of 10 μg/ml the infection titer of the virus descendants (ID_{50}) lowered from 4.8 lg in control to 1.5 lg.

An effect of inhibiting the proliferation of human neoplastic cells under the action of said compounds is established.

The inhibitory concentration (IC_{50}) of the preparation on human neoplastic cells MT-4 and Hep 2 equal to 100 μg/ml was established. It is shown that in this concentration the preparation influences the biosynthetic processes in the cell culture, causing pronounced inhibition of the proliferation of cells and suppression of the synthesis of proteins.

The presence of the preparation in the culture medium in the concentration of 100 μg/ml alters the metabolism of Hep 2 cells, which is manifested in suppression of protein synthesis. Already after 24 hrs of action the disappearance of two proteins is observed: with the molecular mass of 90 kD and 50 kD. After 3 days of follow-up in control the appearance of as new protein with a molecular mass of about 100 kD was noted. The pres-

ence remained on the 6th day as well. In the culture which was under the action of the preparation the synthesis of this protein was absent.

It was shown that the compounds of formula (I) bind to albumins (maximum concentration was 240 μg/ml), and in such form they are easily transported to various organs or tissues. An analysis of data on the tissue accessibility, which characterizes the intensity of penetration of the preparation into peripheral tissues, has shown that the preparation penetrates into all organs. The preparation penetrates most intensively into the liver, spleen, kidneys and lungs. The proposed compounds, unlike other fullerene derivatives that are poorly soluble in water, penetrate only little into the omentum and the brain and do not cause neurotoxicity.

These compounds have a sufficiently low molecular weight for passing through the excretory membrane of cells. Being unlimitedly soluble in water, the elimination of the compounds from the organism is fast enough. Studying the excretion of the preparation has shown that during three days 52—54% of the administered dose is eliminated with urine; the excretion rate in intravenous and rectal administration is the same.

The influence of the preparation on the enzymatic system of biotransformation of xenobiotics in the liver of rats was assessed by variations in the activity of the enzymes: aldehyde dehydrogenase, NADPN-cytochrome-C-reductase, N-demethylase, aniline hydroxylase, obtained from the supermitochondrial fraction of the liver, and in the content of P450 and B5 in rectal administration of the preparation to animals in two doses of 0.3 and 3.0 mg/kg. No dose-dependent effect was revealed on the activity of the studied enzymes and on the content of P450 and B5 in the liver of rats under single administration during 17 days. There was shown a statistically reliable increase of the activity of glutathione transferase in the liver of rats for 1 day after the administration of the preparation as against the placebo-given and intact animals.

The compounds of formula (I) do not have immunotoxic activity. When administered in doses of up to 15 mg/kg, the claimed compounds do not have mutagenic activity with respect t to warm-blooded animals. LD_{50} for nondescript mice was: upon intravenous administration for females — 83 mg/kg, for males — 114 mg/kg; upon intraperitoneal administration — 688 mg/kg for males and females; upon intragastric administration — >2700 mg/kg; and upon rectal administration — 450 mg/kg.

Pharmaceutically ready forms of preparations of the compounds of formula (I) can bed made suitable for oral or parenteral use for the therapy or prophylaxis of virus infections or conditions, in which use of antioxidants and antidotes is indicated.

The compounds are mixed with conventional pharmaceutical carriers and excipients and used in the form of tablets, capsules, suppositories, ointments, solutions for injections, etc. Compositions including compounds of formula (I) contain approximately 0.1—90%, most preferably 0.5—1 0% by weight of the active compound.

The compounds of the present invention can be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intragluteal injections or infusions), by inhalation spraying or rectally, comprising conventional non-toxic pharmaceutically acceptable carriers, stimulants and accessory agents.

Such pharmaceutical compositions can be produced in the form of orally administered suspensions or tablets; nasal sprays; sterile preparations for injections, for instance, in the form of sterile aqueous or oil suspensions for injections, or of suppositories.

For oral administration in the form of suspensions, compositions are prepared by following the procedures widely known in the field of preparing pharmaceutical formulations, and they can contain microcrystalline cellulose to provide weight, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer and sweeteners and/or aromatizers known in this field of the art. In the form of instant release tablets these compositions can contain microcrystalline cellulose, calcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binding agents, expanders, disintegrators, diluents and lubricants known in this field of the art.

For administering in the form of nasal aerosols or by inhalation, such compositions are prepared by the methods well known in the field of pharmaceutical formulations, and they can be produced in the form of solutions based on physiologic saline, with the use of benzoic acid or other suitable preservatives, adsorption promoters for enhancing bioapplicability and/or solubilizing or dispersing agents known in this field of the art.

Solutions or suspensions for injections can be formed in accordance with the known methods, with the use of non-toxic, parenterally applicable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic solution of sodium chloride or suitable dispersing or wetting and suspending agents, such as sterile, soft, stable oils, except for synthetic mono- or diglycerides, or fatty acids, except for oleic acid.

For rectal administration in the form of suppositories, such compositions can be prepared by mixing the drug with such non-irritating excipient as cocoa butter, synthetic

glyceride esters or polyethylene glycols, which are solids at ordinary temperatures but become liquefied and/or dissolve in the rectal cavity with release of the drug.

The proposed compounds can be used for treating infections caused by HIV, HSV, hepatitis C.

Treating infectious diseases by the action of pharmaceutically acceptable doses of compounds of formula (I) is carried out for several viruses simultaneously (in the case of mixed infections) and affects different stages of virus replication. It is shown that the treatment is accompanied by lowering the stress effect to the administration of the preparation, enhancing the antioxidant protection of the organism from infections, eliminating toxins from the organism. Intoxication of the organism is typical of the course of a number of virus infections and is responsible for the gravity of disease.

Estimated data have shown that dosage levels on the order of 0.1—250 or 2500 mg/day can be used for treating or preventing the above-indicated conditions, oral dosages being 2—5 times higher. However, it should be borne in mind that the particular dosage level and the frequency of the drug administration for each particular patient will depend on a large number of factors, including the activity of a particular compound, the metabolic stability and duration of the action, the rate of elimination, the patient's age, body weight, general well-being, sex, drug combinations.

The compounds of formula (I) can be used together with other antivirus agents, immunomodulators, anti-infective agents or vaccines in various combinations with any pharmaceutical formulations intended for treating.

CLAIMS:

1. An agent for inhibiting membrane virus reproduction, characterized in that it comprises a water-soluble compound of fullerene polycarboxylic anions of the general formula

$C_{60}H_n[NH(CH_2)_mC(O)O]_n$,

where C_{60} is the fullerene core,

$NH(CH_2)_mC(O)O^-$ is the aminocarboxylic anion,

m is an integer, preferably 3 and 5, most preferably 5,

n is an integer from 2 to 12, preferably from 4 to 6, most preferably 6.

2. A method for the production of an agent for inhibiting membrane virus reproduction, characterized in that an amino acid in the form of potassium or sodium salt is introduced into an o-dichlorobenzene solution of fullerene, then a solubilizer selected from the group of polyethylene oxides is added: polyethylene glycols with a molecular weight of 150 to 400 and higher, and also dimethyl ethers of polyethylene glycols or 18-crown-6, wherein the amount of the amino acid should be more than 50 times that of fullerene and the synthesis is carried out at a temperature of 60—80°C.

3. A pharmaceutical composition for inhibiting the membrane virus reproduction, characterized in that it contains the agent according to claim 1 in an effective amount and pharmaceutically acceptable fillers.

4. A pharmaceutical composition for inhibiting the membrane virus reproduction according to claim 3, characterized in that it is prepared in the form of tablets, capsules, a solution for injections, suppositories, ointment, a cream, a spray, a gel.

5. A method for inhibiting membrane virus reproduction, characterized in that the pharmaceutical composition according to claims 3 and 4 is used for the suppression of viruses when treating diseases caused by HIV/AIDS, herpes-infections, viral hepatitis C.

Abstract

The present invention relates to the pharmaceutical industry and more particularly to the provision of an agent for inhibiting membrane virus replication.

The technical object of the invention is to provide an agent based on fullerene polycarboxylic anions for suppressing the activity of membrane viruses in treating diseases caused by these viruses.

For accomplishing said subject, there is proposed a group of inventions united by a common inventive concept, said group comprising a method for preparing compounds, studying the mechanisms of action, provision of pharmaceutical compositions, and developing methods of treating with their use.

Said object is accomplished by selecting such quantitative ratios of the components and reaction conditions, which ensure the preparation of polyaddition products. It has been established that in carrying out the synthesis the amount of the amino acid must exceed the amount of fullerene by more than 50 times.

The product produced by the proposed method has an unlimited solubility in water, required bioavailability, high efficiency of action on non-infected cells, low toxicity. The content of the main substance in the target product is at least 87%. The process is adaptable to streamline production and can be used in the pharmaceutical industry.

Compositions of drugs for and methods of treating infectious diseases caused by HIV/AIDS, herpes infections and hepatitis C virus have been developed.

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(516) IPC No.: **7**

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(54) Title: **NARROW POLY- AND MONO-DISPERSED WATER-SOLUBLE OLIGOMER, METHOD OF PREPARING THEREOF, PHARMACEUTICAL AGENT AND METHOD OF INHIBITING ACTIVITY OF VIRUSES**

(56) Analogs of the invention: **EP 0043974 A, 20.01.1982. GB 781479 A, 21.08.1957. FR 2669535 A, 29.05.1992. US 4604404 A, 05.08.1986. SU 905228 A, 15.02.1982.**

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(85) Date of correspondence with Art. 22/39 PCT: **1994.07.08**

(86) Number and date of International or Regional Publication: **US 93/00094 (07.01.1993)**

(87) Number and date of International or Regional Application: **WO 93/14146 (22.07.1993)**

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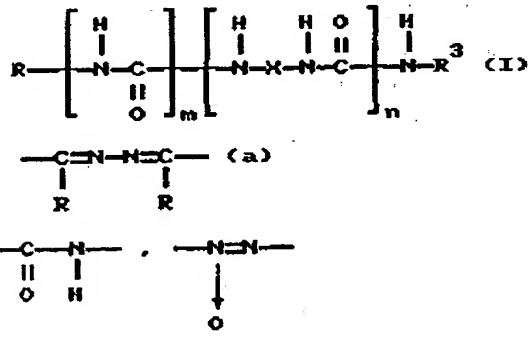
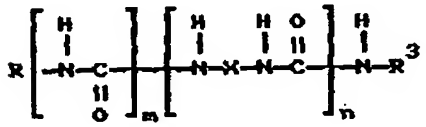
No. 2160746. Abstract

There is described a narrow poly- and mono-dispersed water-soluble oligomer which has a polydispersity ratio from 1.0 to 1.3 and comprises a polyurea of formula (I) wherein R is a hydrogen atom, a C_1-C_4 alkyl group, a phenyl group or a phenyl group substituted by 1—2 fragments R^1 and having up to 3 substituents independently selected from chlorine or bromine atoms or from C_1-C_4 alkyl groups; R^1 is SO_3R^2, $-CO_2R^2$, $-PO_3(R^2)_2$ or $-OPO_3R^2$; R^2 is a hydrogen atom or a pharmaceutically acceptable cation; m = 0 or 1, provided that if m = 0, R is a hydrogen atom; X is an aromatic substituent as defined in claim 1; Y is $-CO_2-$, -C=C-, -N=N- or (a) n is an integer from 3 to15; R^3 is R or $-X-NH_2$ where R and X are as defined above. The compounds have biological activity against the human immunodeficiency virus. A pharmaceutical preparation based thereon and a method of preparing the oligomer, and a method of inhibiting the activity of viruses are also described. 6 independent and 20 dependent claims, 15 dwgs, 3 tbls.



No. 2160748. Claims

1. A narrow poly- or mono-dispersed water-soluble oligomer which has a polydispersity ratio of 1.0 to 1.3 and which comprises a polyurea of formula (I)



wherein

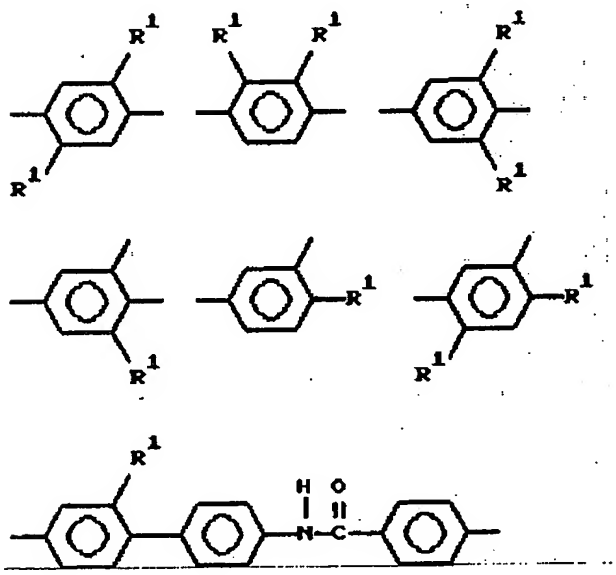
R is a hydrogen atom, a C_1-C_4 alkyl group, a phenyl group or a phenyl group substituted by 1—2 fragments R^1 and having up to 3 substituents independently selected from chlorine or bromine atoms or from C_1-C_4 alkyl groups:

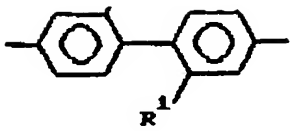
R^1 is SO_3R^2, $-CO_2R^2$, $-PO_3(R^2)_2$ or $-OPO_3R^2$;

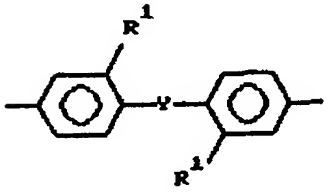
R^2 is a hydrogen atom or a pharmaceutically acceptable cation;

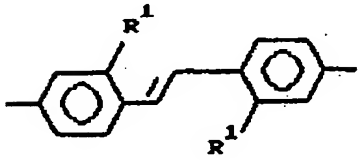
m = 0 or 1, provided that if m = 0, R is a hydrogen atom;

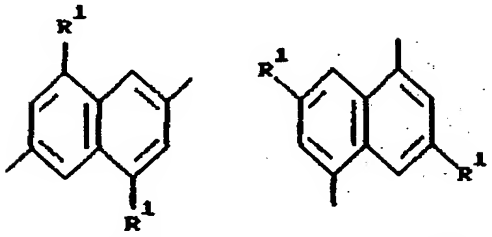
X is



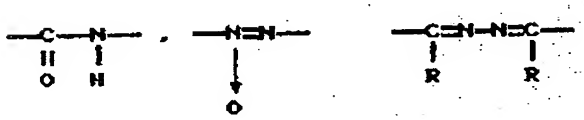








where Y is $-CO_2-$, -C=C-, -N=N-,



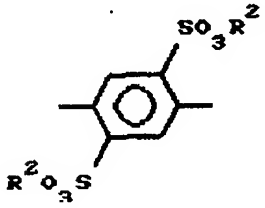
n is an integer from 3 to 15,

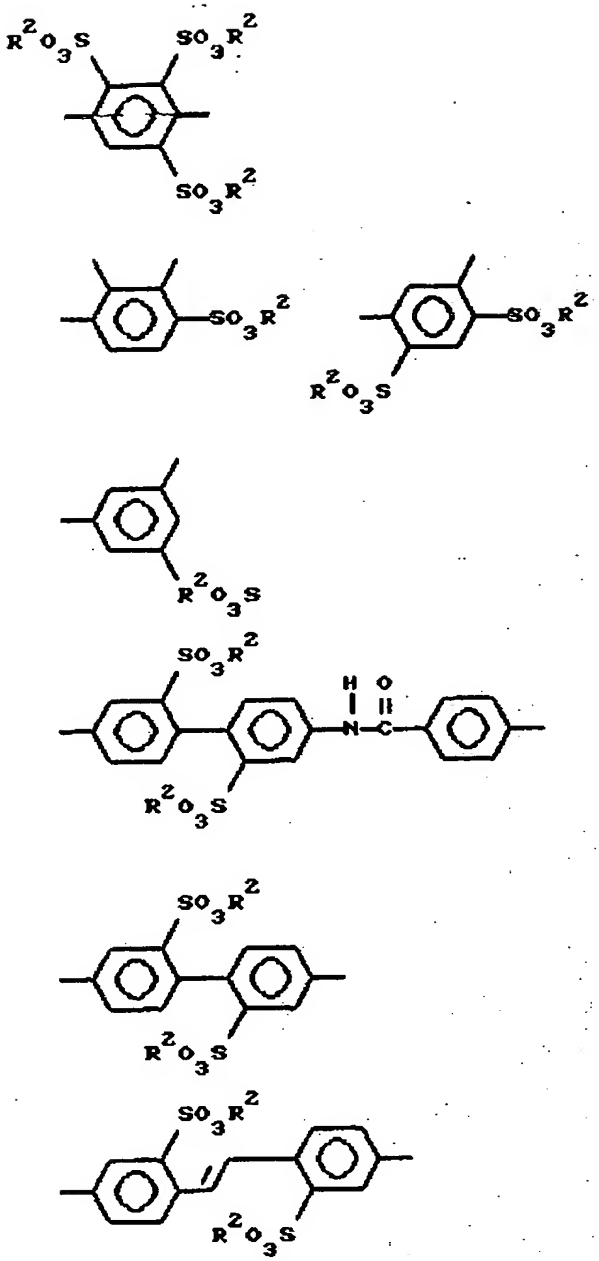
R^3 is –R or $-X-NH_2$ where R and X are as defined above.

2. The oligomer of claim 1 where n is an integer from 5 to 10.

3.The oligomer of claim 2 where n is an integer from 6 to 9.

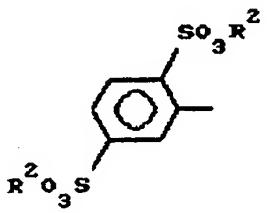
4. The oligomer of claim 1, which is a polyurea of formula (I) where n is an integer from 3 to 125 and X is



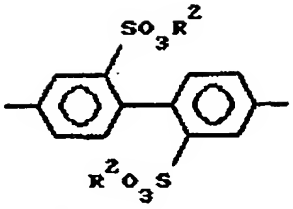


R^2 is as defined in claim 1.

5. The oligomer of claim 4 where R and R^3 denote 4-methylphenyl, R^2 is hydrogen, X is



6. The oligomer of claim 4 where X is



R^2 is the same as in claim 1.

7. The oligomer of claim 4 where R is a phenyl group substituted with from 1 to 2 R^1 moieties and up to 3 bromo substituents.

8. The narrow poly-dispersed oligomer of claims 1, 4 and 7, which has a polydispersity ratio of from 1.0 to 1.2.

9. The mono-dispersed oligomer of claims 1, 4 and 7, which has a polydispersity ratio of from 1.0 to 1.1.

10. The mono-dispersed oligomer of claim 7, which has the polydispersity ratio of 1.05.

11. A pharmaceutical preparation which inhibits the activity of HIV-1 and HSV-2 viruses, characterized in that it contains a pharmaceutically effective amount of the narrow poly- or mono-dispersed oligomer of any one of claims 1 to 10 with a pharmaceutically acceptable carrier.

12. A method of inhibiting the activity of HIV-1 and HSV-2 viruses in vitro with the help of a virus activity inhibitor, characterized in that as the virus activity inhibitor use is made of the pharmaceutical preparation of claim 11 in an effective amount.

13. A method of preparing the narrow poly- and mono-dispersed anionic oligomers of claim 1, comprising the steps of limiting a corresponding non-purified poly-dispersed mixture of anionic oligomers by gel filtration, selective precipitation, membrane separation or reversed-phase chromatography, which as a result give the desired polydispersity ratio of 1.0 to 1.3.

14. The method of claim 13, characterized in that it comprises a further step of converting a narrow poly-dispersed anionic oligomer salt to the desired pharmaceutically acceptable salt.

15. The method of claim 13, characterized in that the polydispersity ratio of the oligomer is1.0 to 1.2.

16. The method of claim 13, characterized in that the polydispersity ratio of the oligomer is 1.0 to 1.1.

17. The method of preparing narrow poly- and mono-dispersed anionic oligomers of claim 1, consisting in that the mono-dispersed anionic oligomer is isolated by gel electrophoresis or reverse-phase chromatography.

18. The method of claim 17, characterized in that it comprises a further step of converting a narrow poly-dispersed anionic oligomer salt to the desired pharmaceutically acceptable salt.

19. The method of claim 17, characterized in that the purity of the mono-dispersed material is at least 75%.

20. The method of claim 17, characterized in that the purity of the mono-dispersed fraction is from about 85 to about 100%.

21. A method of preparing the narrow poly- and mono-dispersed anionic oligomers of claim 1, comprising the steps of limiting a corresponding non-purified poly-dispersed mixture of anionic oligomers by gel filtration, selective precipitation, membrane separation or reversed-phase chromatography, which as a result give the desired polydispersity ratio of 1.0 to 1.3; isolating the mono-dispersed anionic oligomer by gel electrophoresis or reverse-phase chromatography.

22. The method of claim 21, characterized in that comprises a further step of converting a narrow poly- or mono-dispersed anionic oligomer salt to the desired pharmaceutically acceptable salt.

23. The method of claims 13, 14, 17, 18, 21 and 22, characterized in that the oligomer is a polyurea of formula (I) and n denotes a monodisperse fraction 5—10

24. The method of claim 23, characterized in that the oligomer is a polyurea of formula (I) and n denotes a monodisperse fraction 6—9.

25. The method of claims 14, 18 and 22, characterized in that the salt is an ammonium, sodium or potassium salt.

26. The method of claims 14, 18 and 22, characterized in that the further step is carried out by ion exchange or by adding of a weak volatile acid salt.

Table I

MTT assay

Fraction n =	M_n	IC_{50} μg/ml	IC_{50} μM
2	976	>2	>24
3	1344	>2	>14
4	1712	>2	>11.6
5	2080	1.8	0.86
6	2448	0.88	0.35
7	2816	0.72	0.25
8	3184	0.7	0.21
9	3552	0.7	0.19
10—13	>3920	1.7	~0.42
Polydisperse	2448	1.1	~0.44

Table II

Determination of syncytium formation

Fraction n =	IC_{50} µg/ml	IC_{50} µM
2	9	9.2
3	7.8	5.8
4	1.9	1.1
5	0.35	0.17
6	0.25	0.10
7	0.15	0.05
8	0.22	0.07
9	0.14	0.04
10—13	0.36	0.09
Polydisperse	0.18	0.07

Table III

Type II

Fraction n =	IC_{50} μg/ml
2	>10
3	<10
4	<10
5	<10
6	<1
7	<1
8	<1
9	<1
10—13	<1

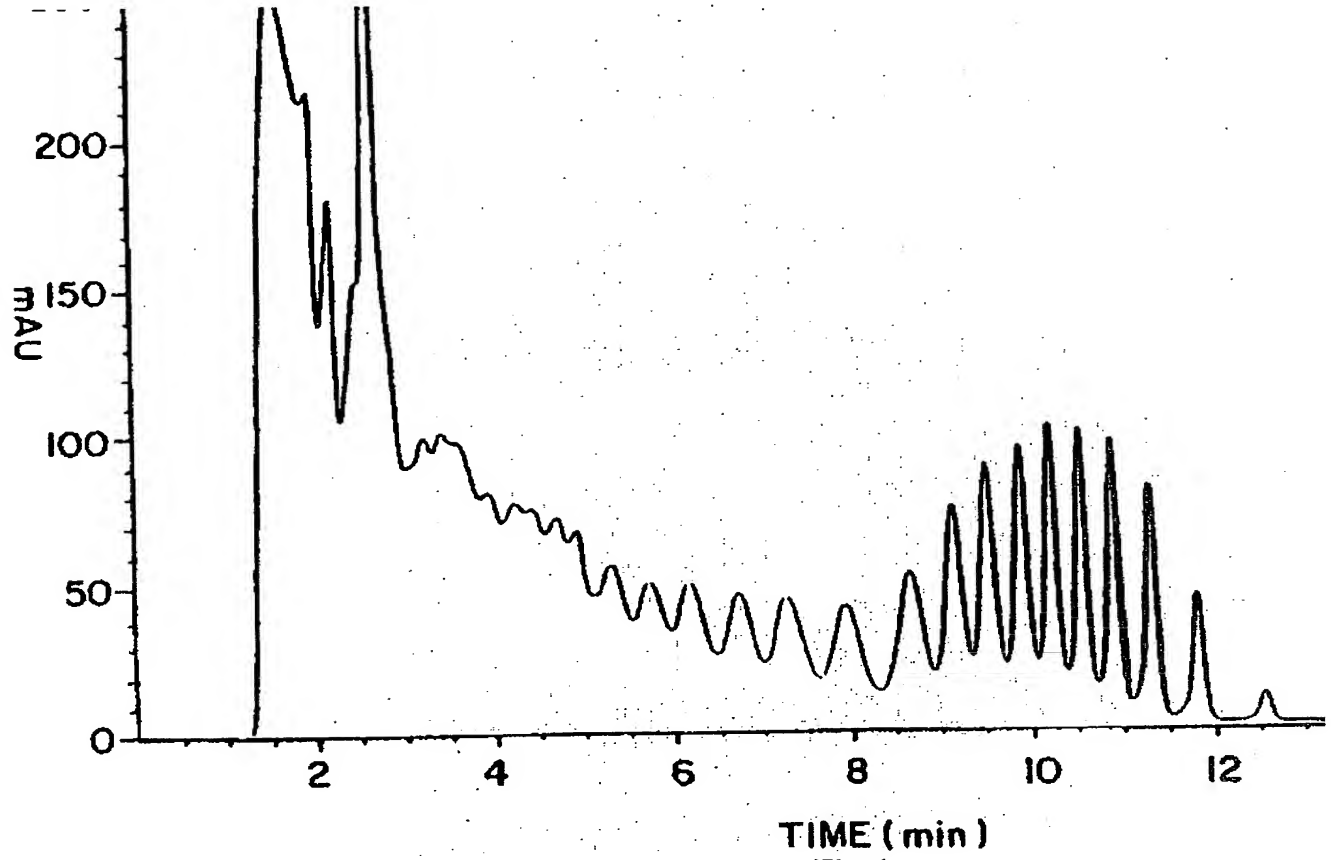


Fig. 1

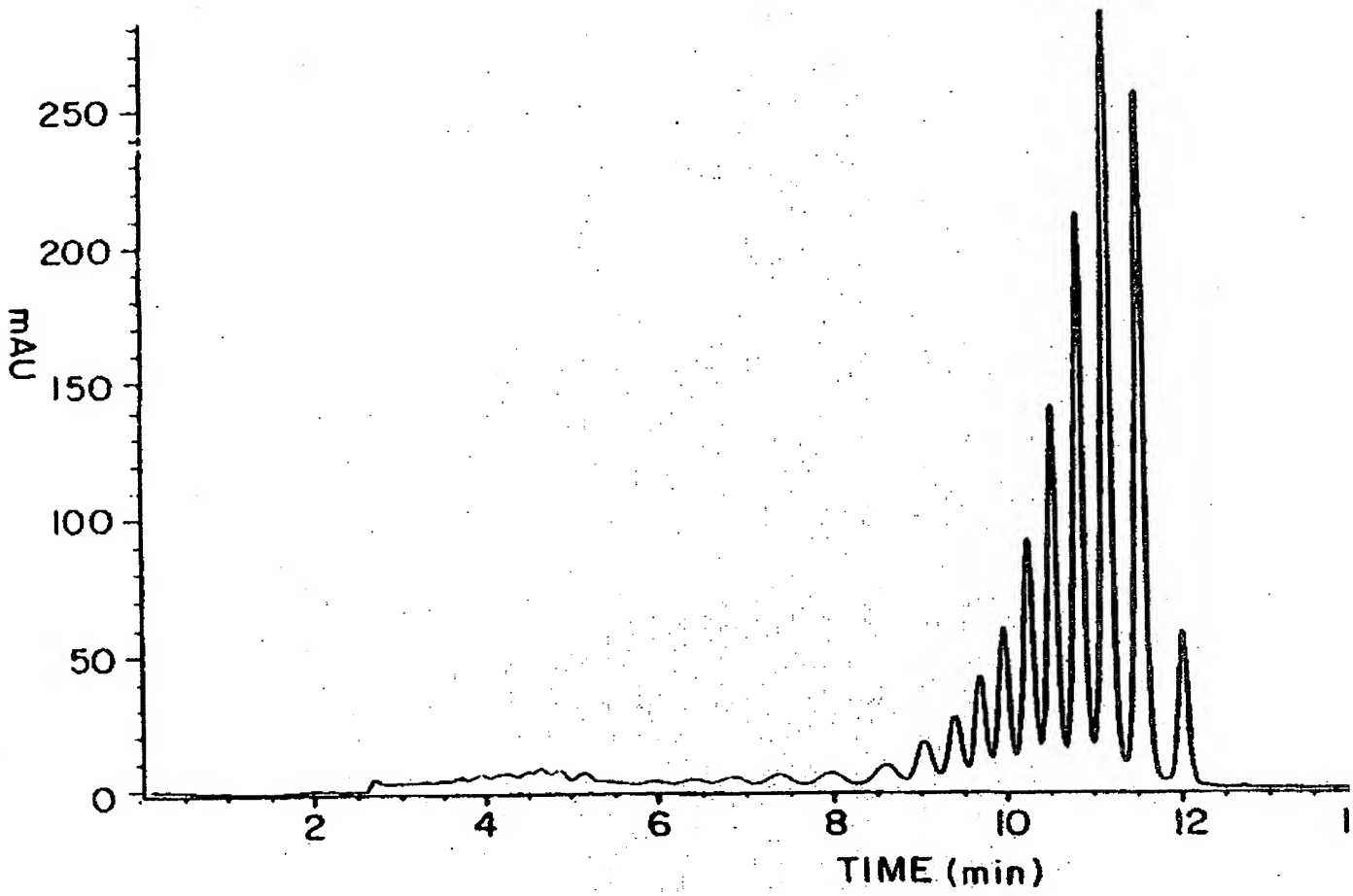


Fig. 2

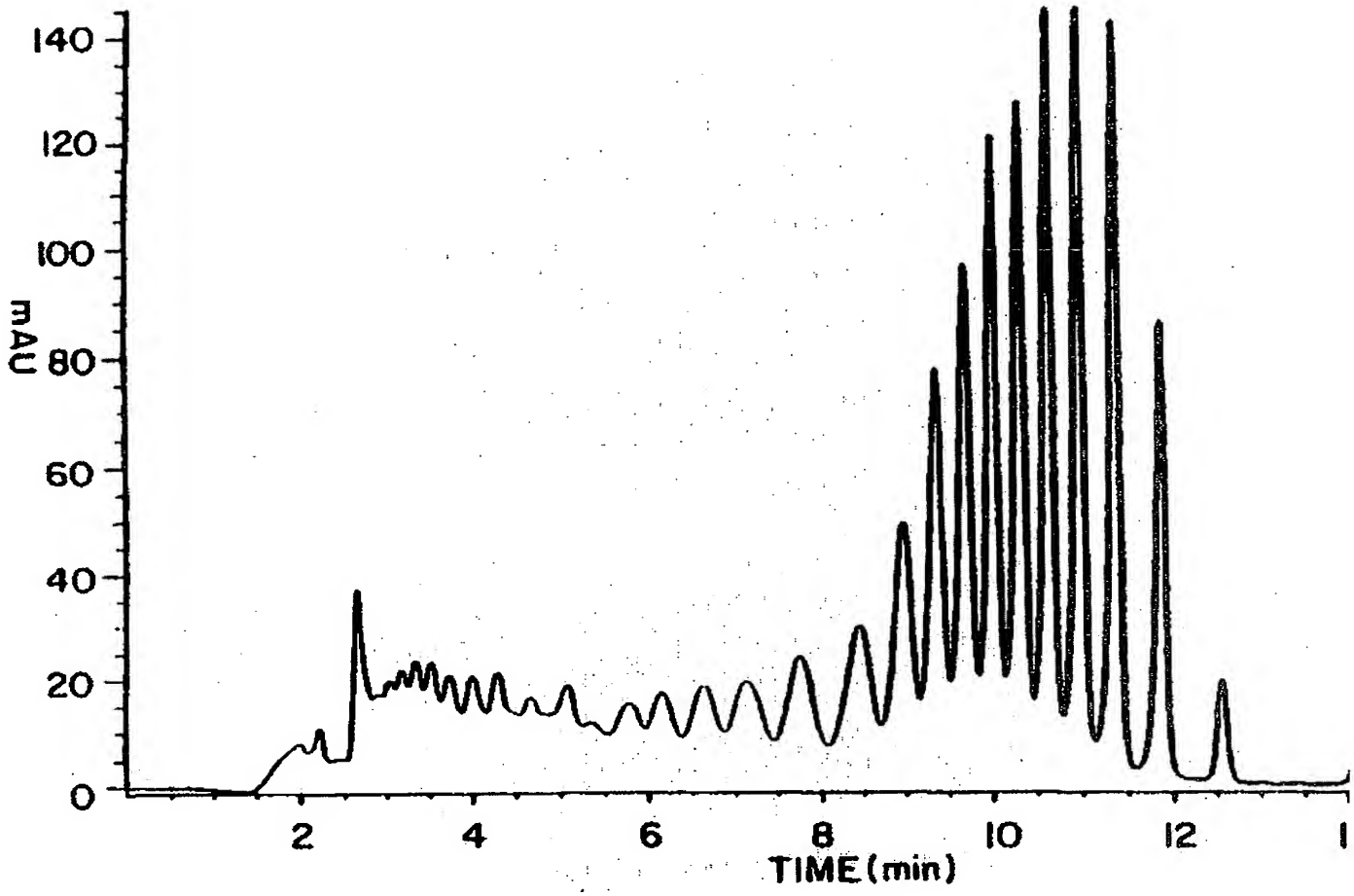


Fig. 3

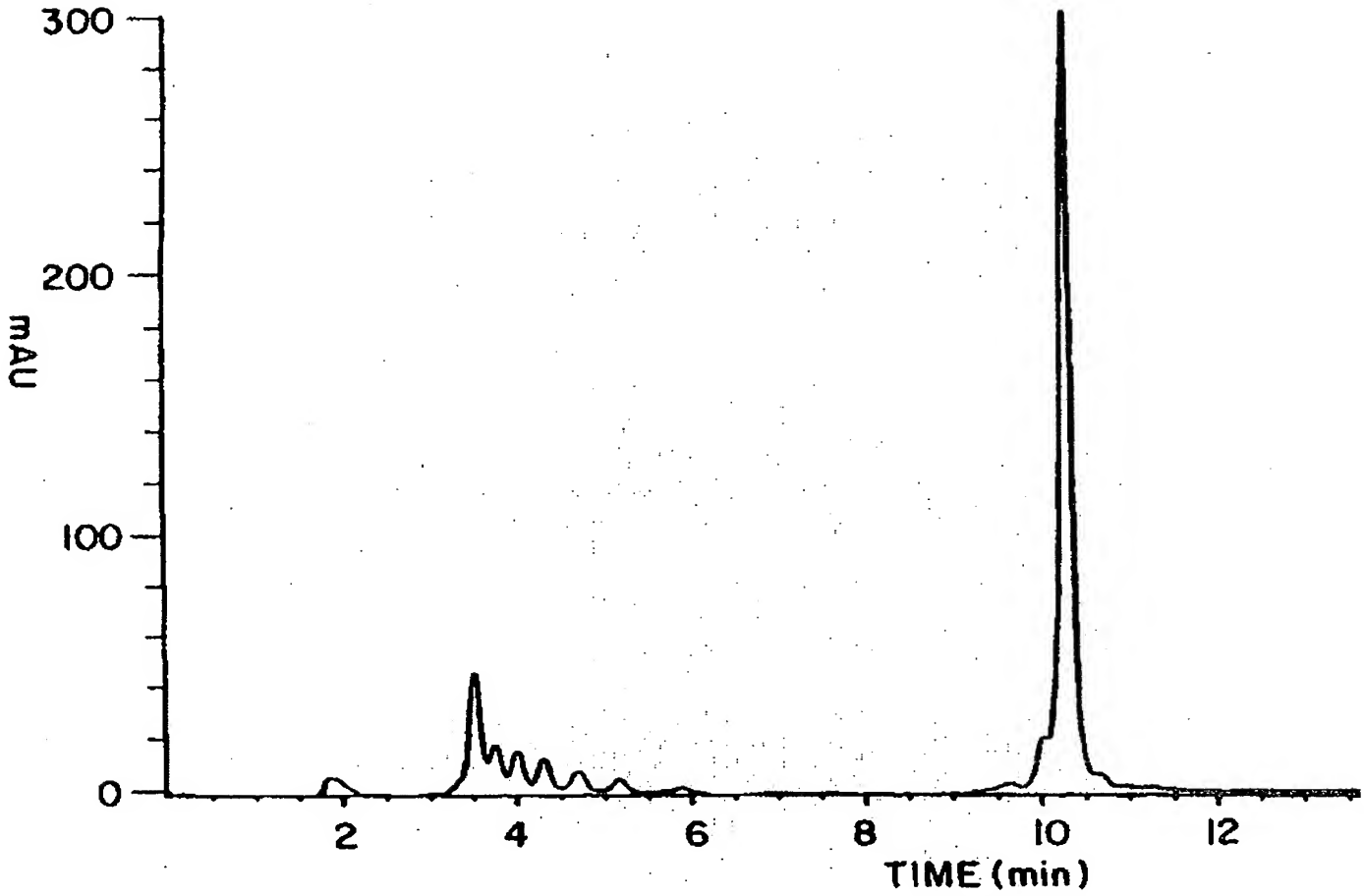


Fig. 4

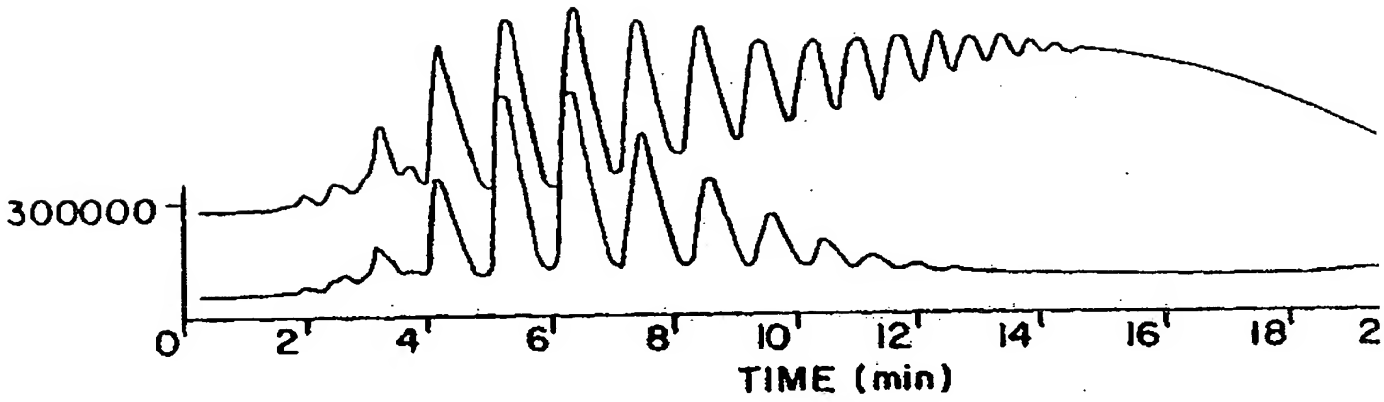


Fig. 5

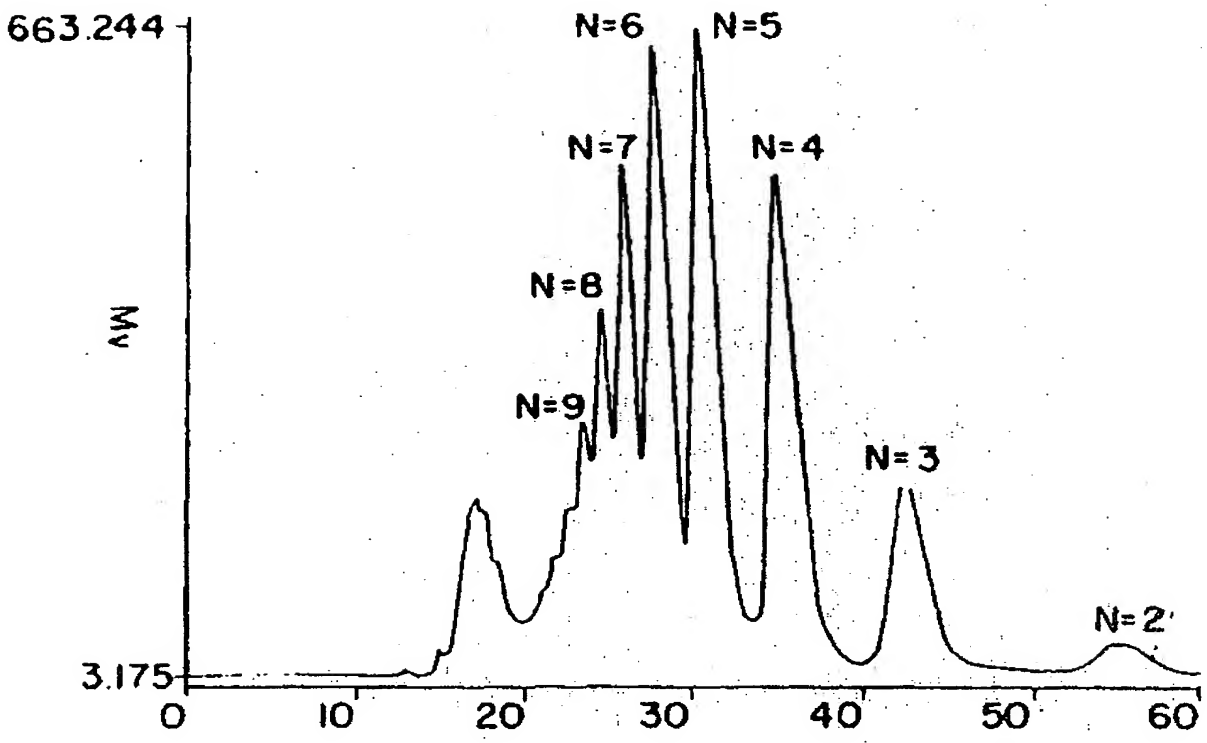


Fig. 6

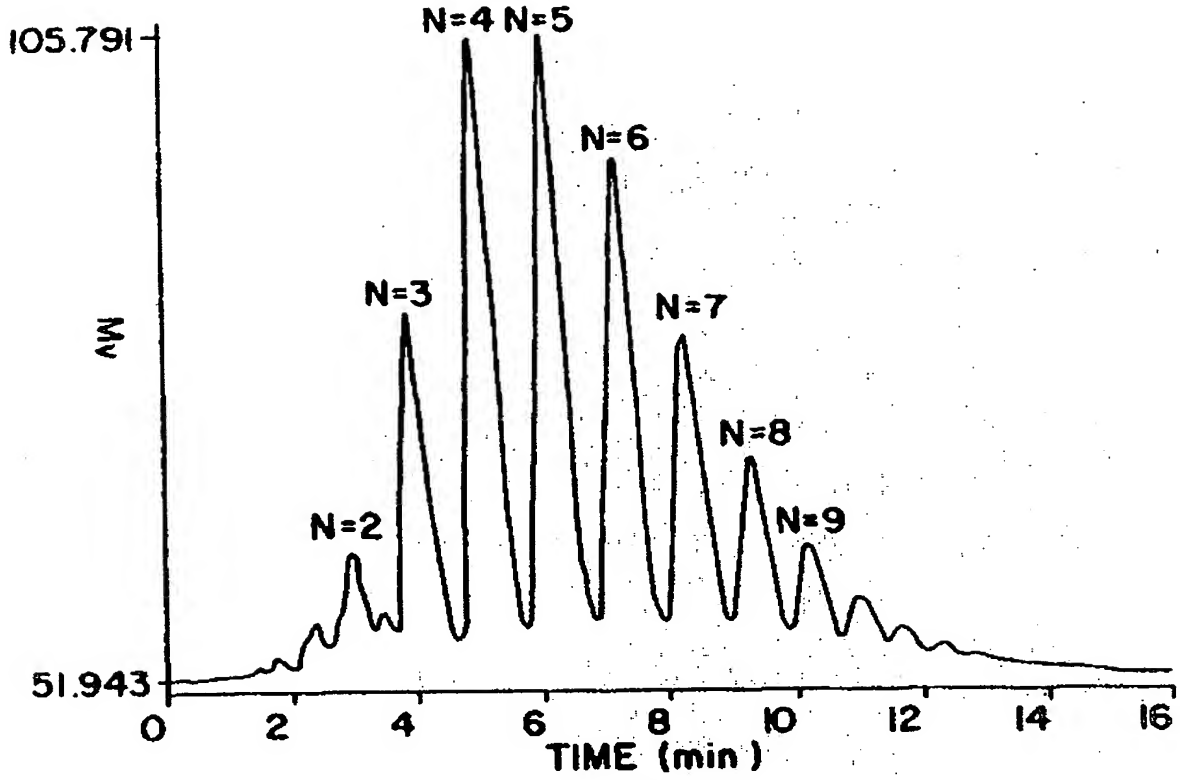


Fig. 7

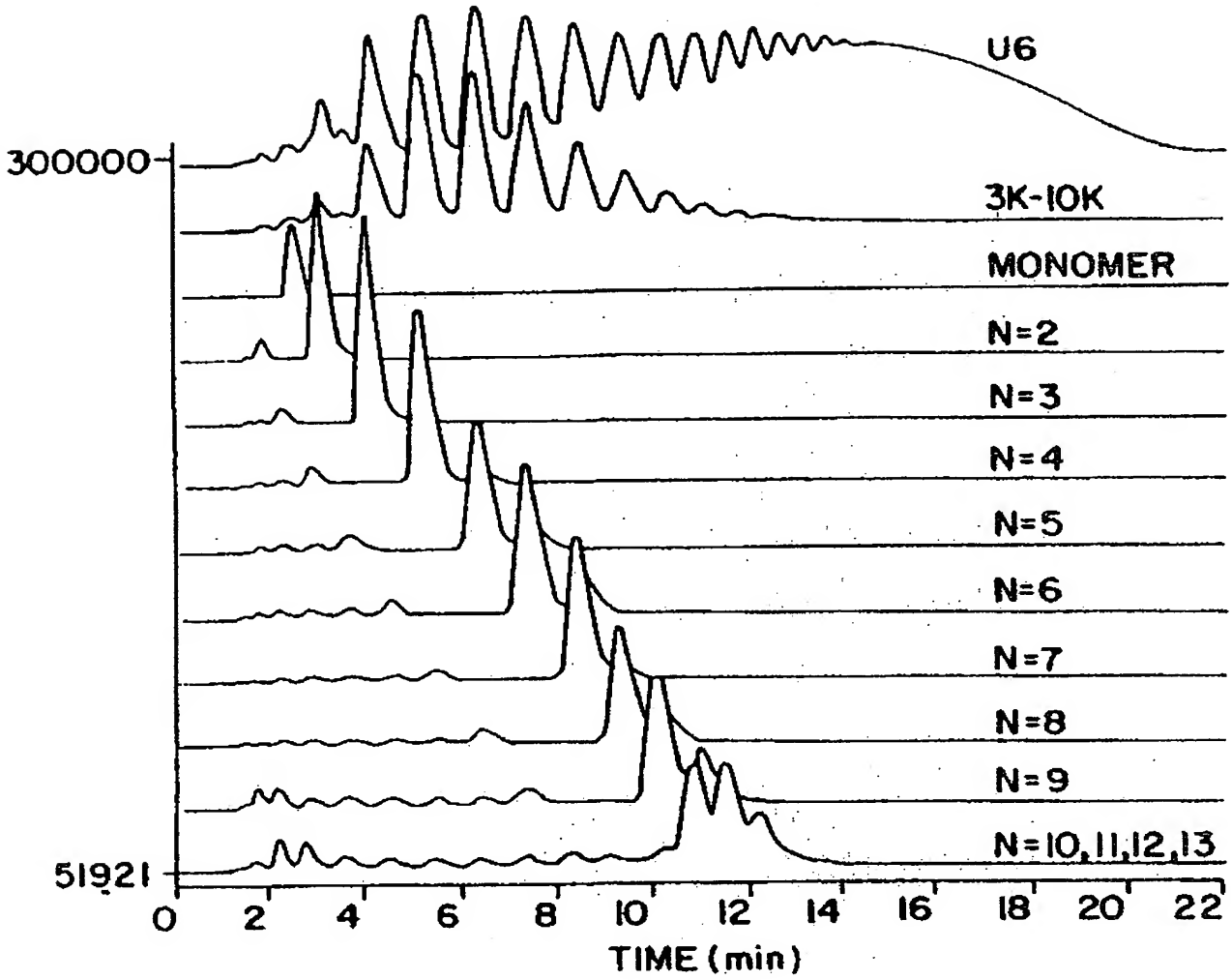

U6
300000
3K-10K
MONOMER
N=2
N=3
N=4
N=5
N=6
N=7
N=8
N=9
N=10,11,12,13
51921
0
2
4
6
8
10
12
14
16
18
20
22
TIME (min)

Fig. 8

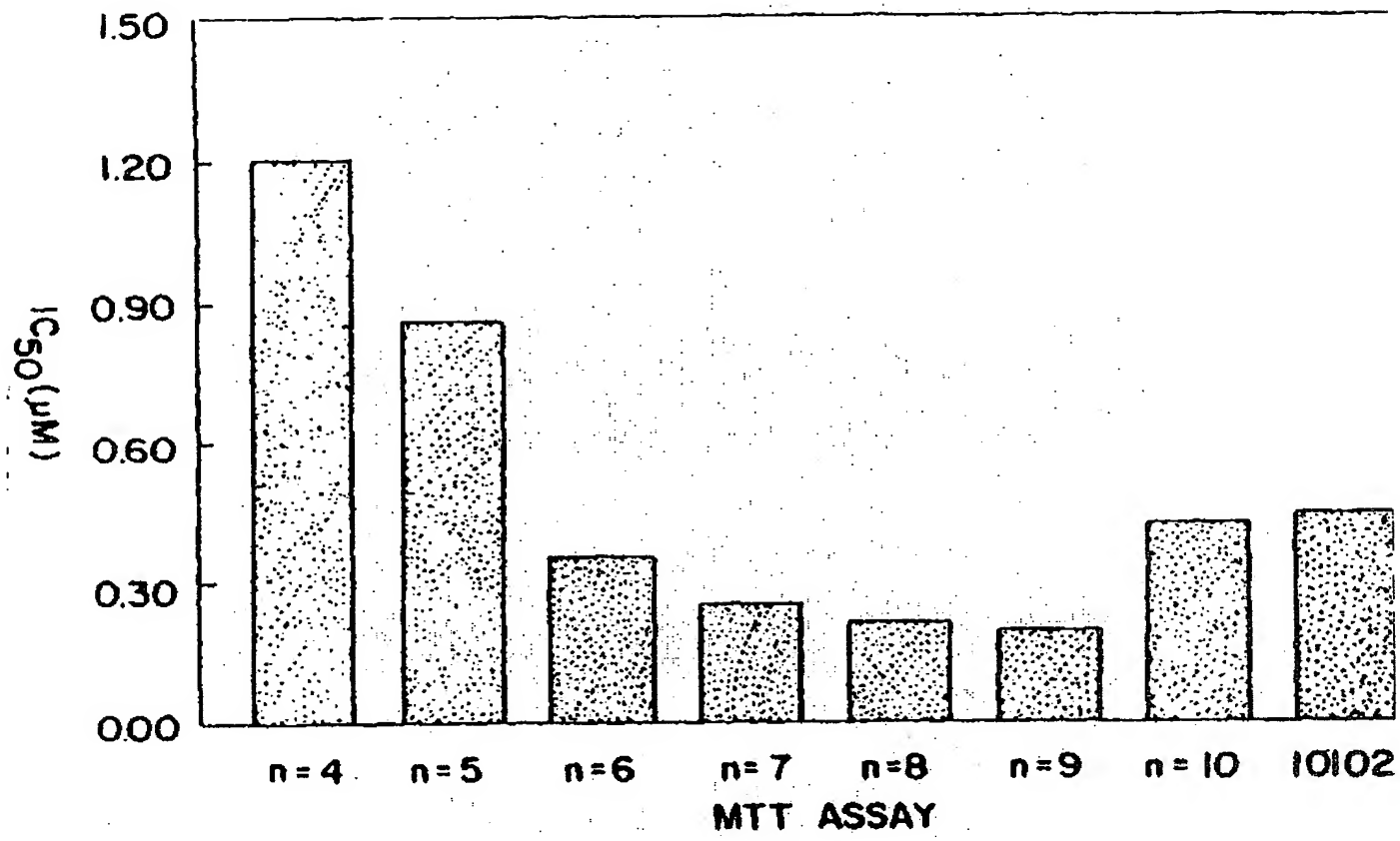

1.50
1.20
0.90
0.60
0.30
0.00
IC50(μM)
n=4
n=5
n=6
n=7
n=8
n=9
n=10
10102
MTT ASSAY

Fig. 9

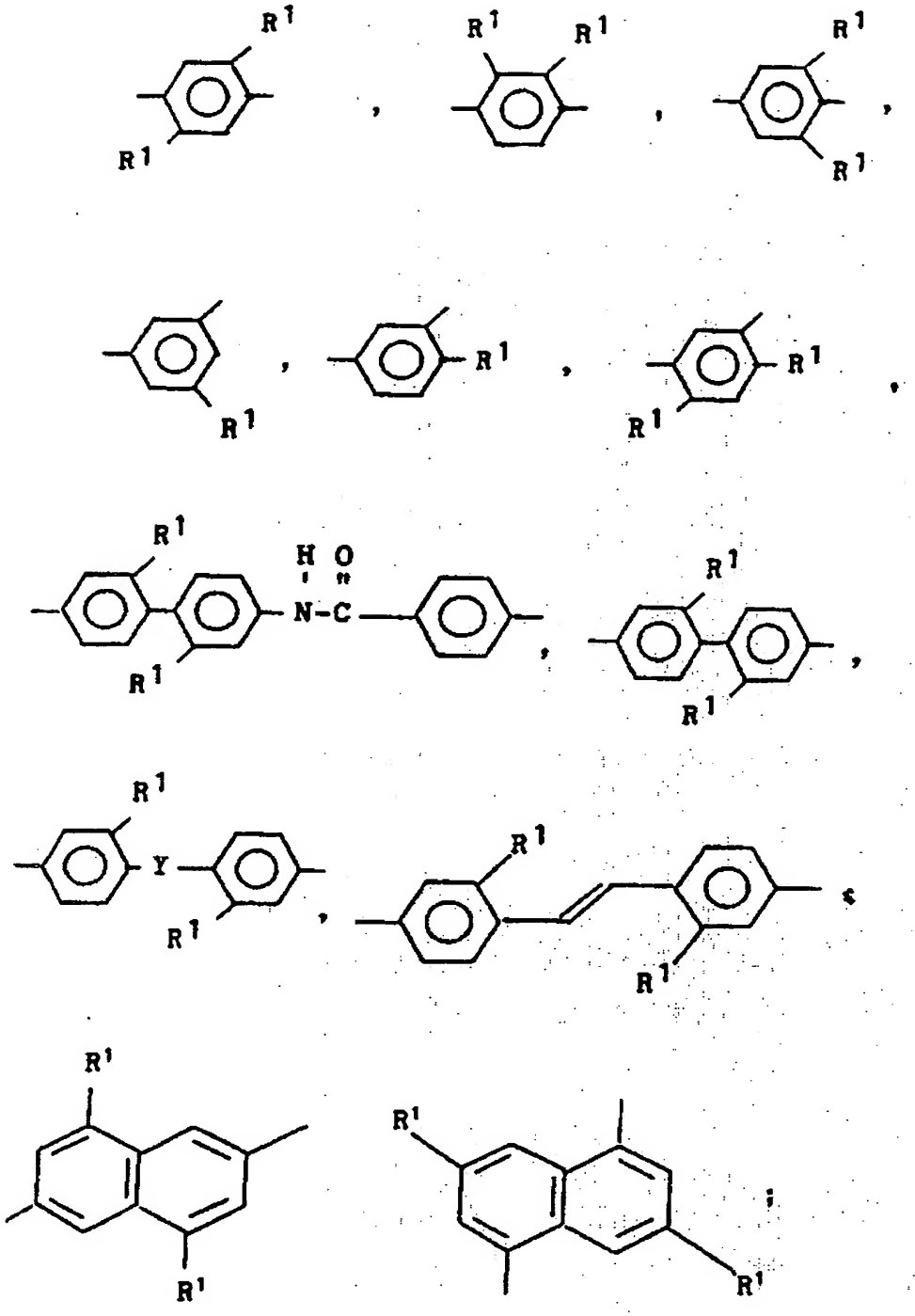


Fig. 10

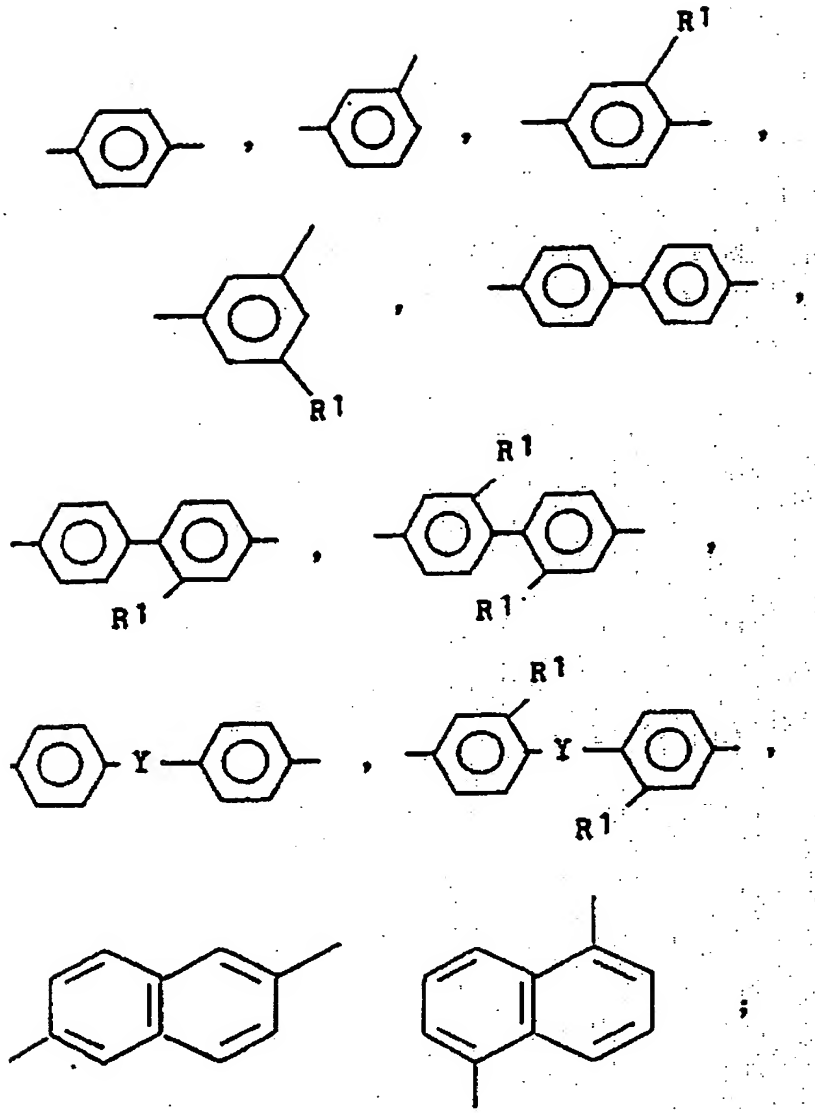
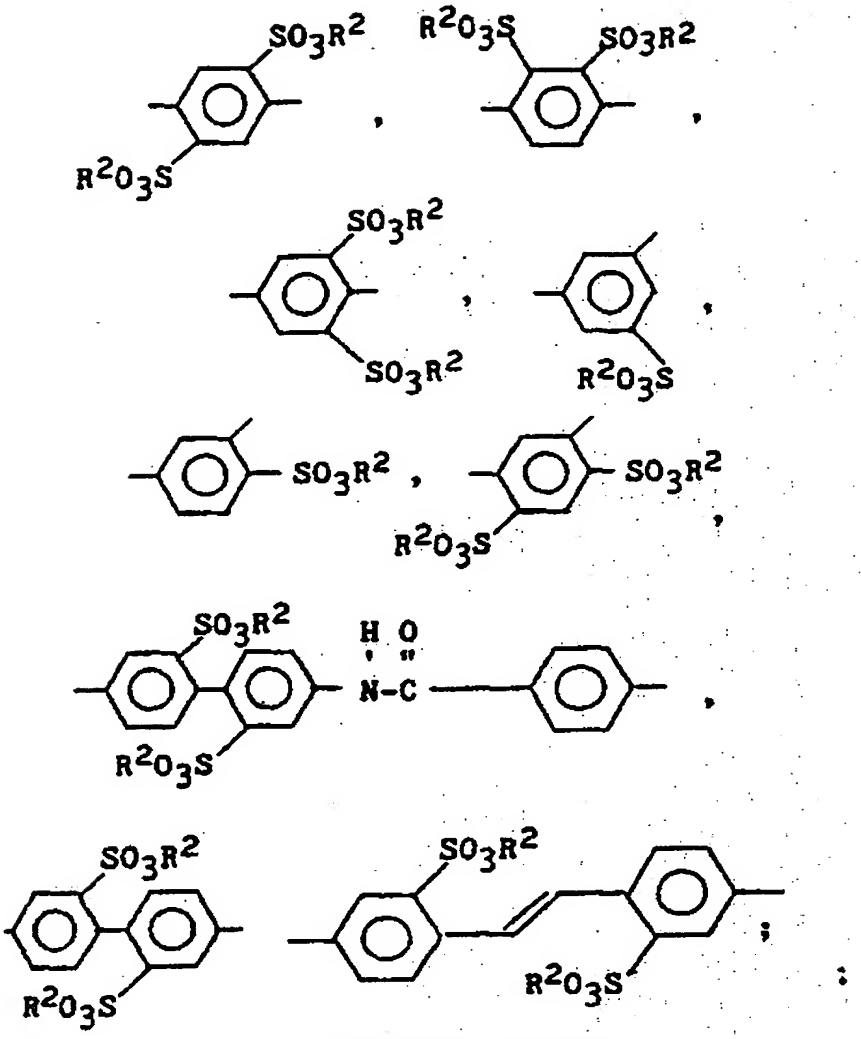


Fig. 11



Particularly preferable

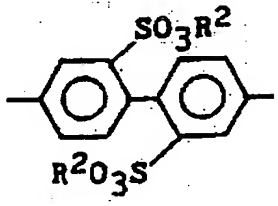


Fig. 12

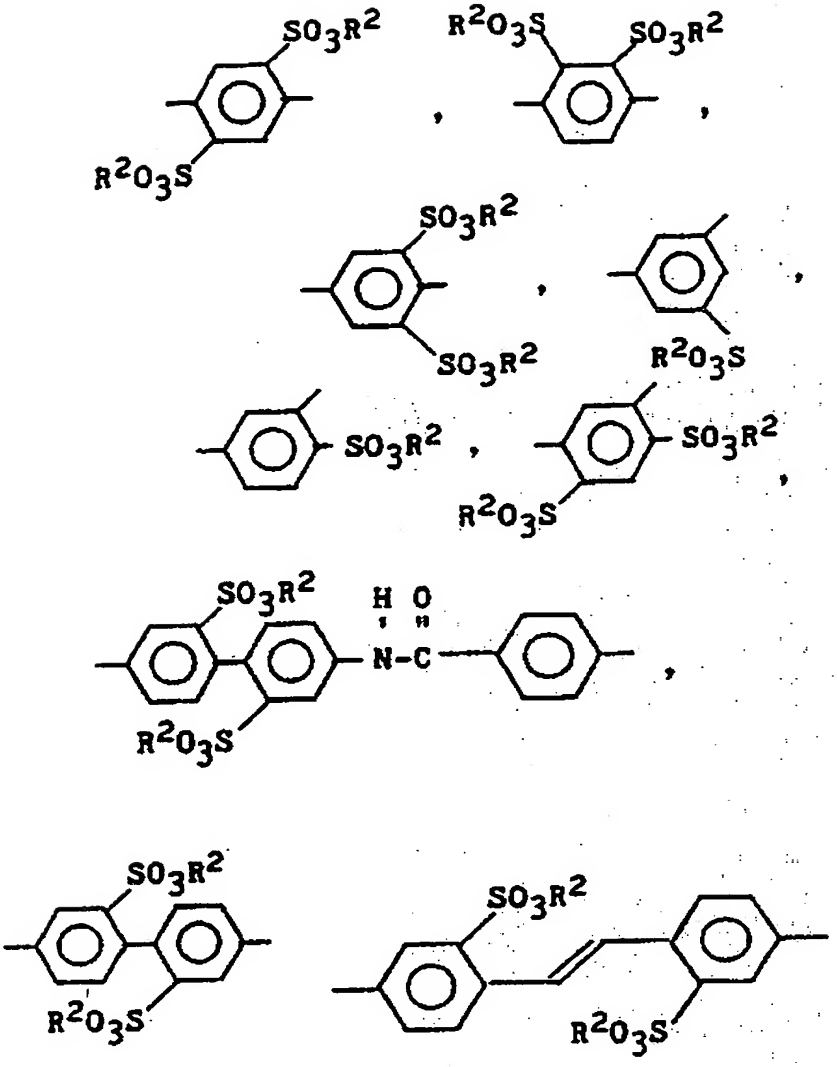

SO3R2
R2O3S
SO3R2
R2O3S
SO3R2
SO3R2
SO3R2
R2O3S
SO3R2
SO3R2
R2O3S
SO3R2
H O
N-C
R2O3S
SO3R2
R2O3S
SO3R2
R2O3S

Fig. 13

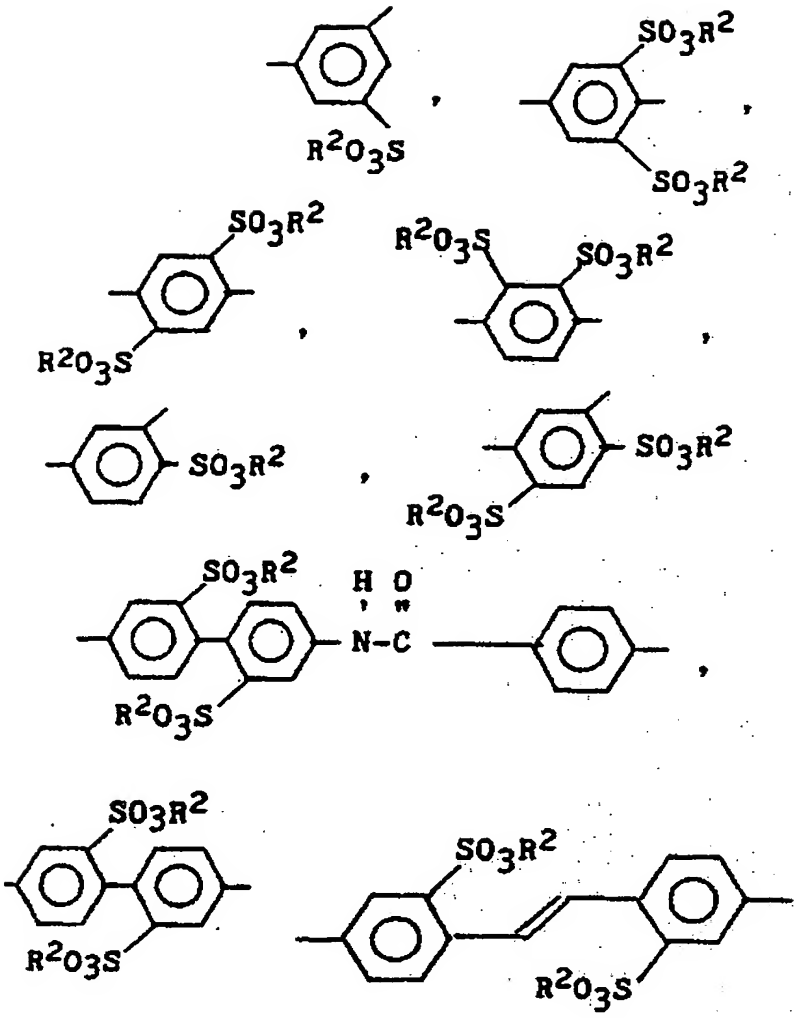


Fig. 14

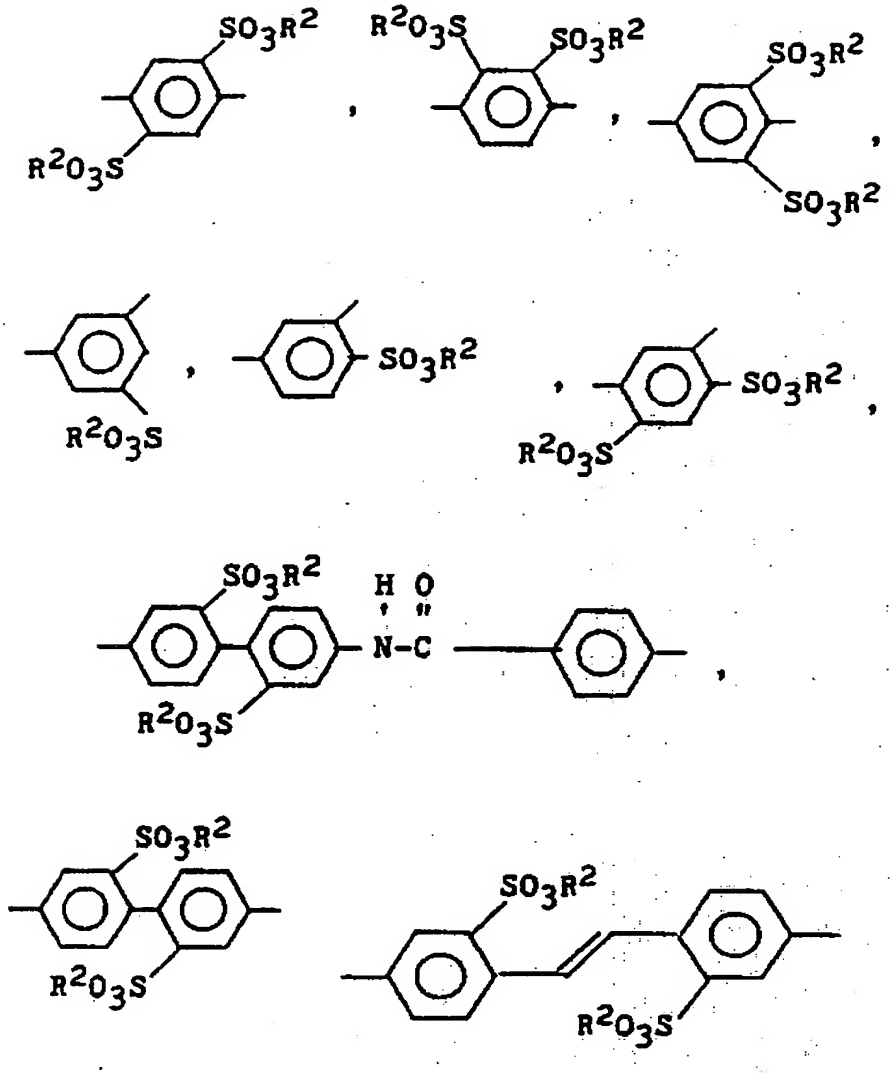


Fig. 15

RU 2162819 C2

No. 2162819. Abstract

The invention relates to organic chemistry, namely, to the chemistry of fullerenes. The method of preparing water-soluble fullerene derivatives comprises mixing fullerenes predissolved in an organic solvent with a polymer matrix in chloroform, evaporating the mixture under4 vacuum till complet5e removal of the solvents, dissolving the resulting complex in a phosphate-salt buffer (pH 7.4—7.6), followed by treating the product with ultrasound, wherein as the water-soluble polymer matrix use is made of membrane cephalins which differ from other phospholipids in that they are soluble both in water and in organic solvents. The technical result is an improvement of the characteristics of aqueous solutions of fullerenes, which are expressed in an enhanced stability of suspension under storage, better membranotropicity of fluorine complexes, their ability of being epodestroyed and biotransformed in the cell. 4 claims, 2 dwgs.

No. 2162819. Claims

1. A method of preparing water-soluble fullerene derivatives comprises mixing fullerenes predissolved in an organic solvent with a polymer matrix in chloroform, evaporating the mixture under4 vacuum till complet5e removal of the solvents, dissolving the resulting complex in a phosphate-salt buffer (pH 7.4—7.6), followed by treating the product with ultrasound, characterized in that as the polymer matrix for fullerenes use is made of naturally occurring phospholipids — cephalins, benzene being used as the solvent of fullerenes in the first step of the process.

2. A method according to claim 1, characterized in that for dissolving fullerene complexes use is made of an aqueous phosphate-salt buffer (ph 7.4-7.6).

3. A method according to claim 1, characterized in that in the final step of the process the solution is treated with ultrasound with the frequency of 22 kHz, intensity of 10 to 60 W/cm^2 for 30 to 60 s.

4. A method according to claim 1, characterized in that the entire process is carried out in the atmosphere of nitrogen.